

# Fall 2019 Volume 24: Number 4

# LabLink

**Director, Bureau of Laboratories  
Sandip Shah, PhD, HCLD(ABB)**

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## **Bureau Vision**

The Bureau of Laboratories is a stronger, more diverse team within an integrated public health system. We utilize advanced technology and innovative leadership to provide comprehensive public health services in our dynamic global community.

## **Bureau Mission**

We are dedicated to continuing leadership in providing quality laboratory science for healthier people and communities through partnerships, communication and technical innovation.





## Eastern Equine Encephalitis -"EEE"

**Author: Jessica Jenkins, MLS(ASCP), Kristin Smith, MT(ASCP), and Diana Riner, Ph.D.**

The Arbovirus season in Michigan is in full swing. When people think of mosquito-borne diseases, West Nile virus is usually what comes to mind; most people have never heard of a virus called Eastern Equine Encephalitis virus, usually referred to as "EEE" or "triple E". Though it is not as well known, EEE has been present in Michigan for decades. According to the Centers for Disease Control and Prevention (CDC), human cases of neuroinvasive EEE are sporadic, with only seven cases reported from 2009-2018 in Michigan.

Eastern Equine Encephalitis virus is a mosquito-borne alphavirus that infects birds, humans, and other mammals, such as horses and deer. The CDC states that birds are the primary host for the EEE virus, while humans and other mammals are incidental, dead-end hosts. The main mosquito vector of EEE virus is *Culiseta melanura*, which feeds on birds. Infections in mammals occur as a result of "bridge vectors." A bridge vector is a mosquito that is capable of transmitting the EEE virus and also feeds on humans. In Michigan, this includes *Coquillettidia perturbans* and potentially, *Aedes* and *Culex* species. Humans and horses are considered to be dead-end hosts because the level of viremia is insufficient for further viral transmission.

Educational materials on [Michigan.gov](https://www.michigan.gov) indicate that only 5% of people infected will develop clinical symptoms, but 1% will have severe disease, involving encephalitis or meningitis, and approximately 33% of symptomatic patients will die. Symptoms of EEE vary from fever, headache, and fatigue to seizures and coma. Those that recover can be

left with debilitating neurological sequelae. The highest rates of neuroinvasive EEE are seen in persons younger than 15 or greater than 50 years old. There is no licensed vaccine to prevent EEE infections in humans, but, there is a vaccine to help protect horses, in which EEE is nearly always fatal.

The Michigan Department of Health and Human Services (MDHHS) weekly summary for arbovirus activity ([https://www.michigan.gov/documents/emergingdiseases/Weekly\\_Arbovirus\\_Summary\\_10-2-2019\\_667548\\_7.pdf](https://www.michigan.gov/documents/emergingdiseases/Weekly_Arbovirus_Summary_10-2-2019_667548_7.pdf)), has reported to date (October 3rd, 2019) nine human cases of EEE in Michigan, which included four deaths. All human cases have been in southwest Michigan, but, 33 EEE positive animals have been identified in 15 counties including counties in central and southeast Michigan as well. The MDHHS Bureau of Laboratories (BOL) provides arbovirus testing for the State of Michigan. The arbovirus panel at the BOL includes the West Nile virus (WNV), St. Louis Encephalitis virus (SLE), EEE, and California Group virus-Lacrosse antigen (CGV). Two types of tests are performed: IgM testing, and if indicated, plaque reduction neutralization testing (PRNT). As antibodies are produced against one arbovirus, they can weakly bind to other arboviruses and "cross-react." Both tests to detect IgM antibodies and neutralizing antibodies are often needed for confirmation of infection.

Two different assays are used for the detection of IgM antibodies. The first assay is called a microsphere immunoassay (MIA). This assay utilizes beads coated with antigens of West Nile virus, St. Louis Encephalitis virus, and Eastern Equine Encephalitis virus. The beads are combined with patient specimens and a fluorescent conjugate. The patient sample/ bead mixture is passed through lasers which classify and quantify the beads to determine if antibodies to WNV, SLE, or EEE are present in the sample. The second IgM test is called an antibody capture enzyme-linked immunosorbent assay (ELISA), in which

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# Eastern Equine Encephalitis -"EEE"

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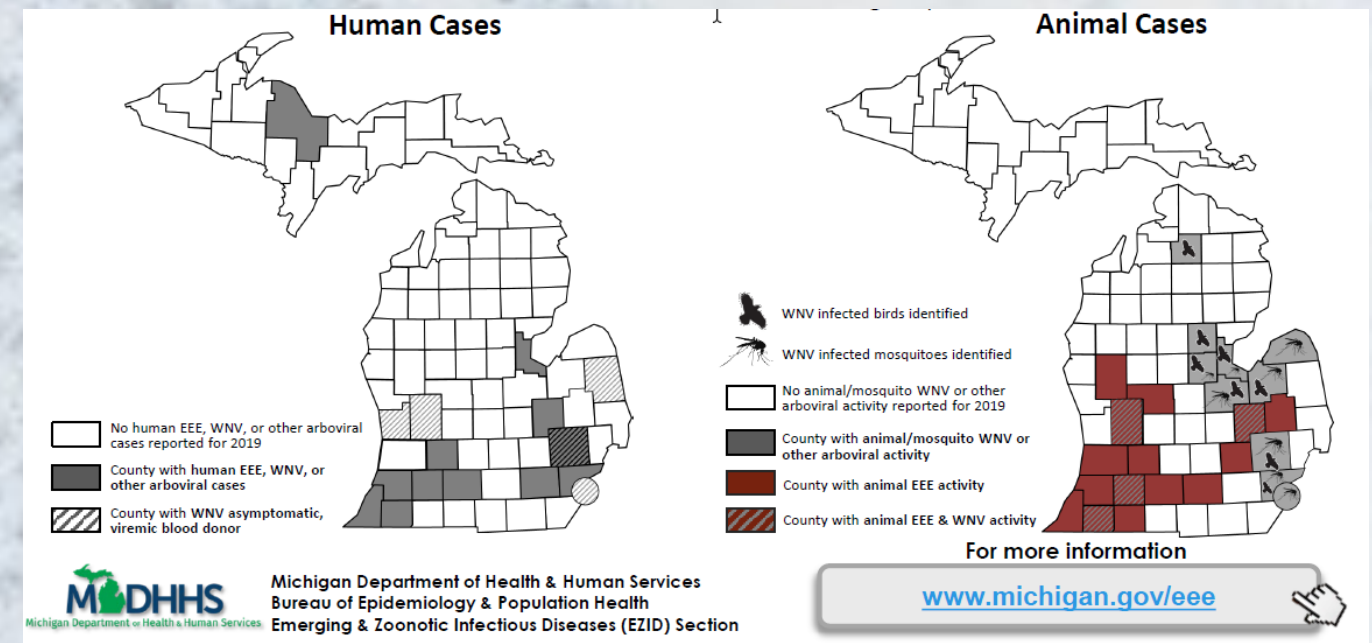
patient specimens are added to a test plate coated with anti-human IgM antibodies then combined with antigens characteristic of CGV. A conjugate and chromogenic substrate produce a color change in positive specimens which is measured by a spectrophotometer.

The PRNT is based on the ability of antibodies in the patient specimen to neutralize the live virus. Specimens are incubated with the virus in question (WNV, SLE, EEE, CGV), then added to a plate with cells. If the virus is not neutralized, it will infect the cells and cause the formation of plaques, which can be counted under a microscope. PRNT is considered to be the confirmatory test for the presence of antibodies. At the BOL, PRNT is performed on all EEE and SLE positive samples, the first WNV or CGV positive sample in a given arbovirus season, and any WNV/SLE uninterpretable sample result. While the ELISA and MIA tests are specific for IgM antibodies, PRNT cannot distinguish between IgM and IgG antibodies. Instead, it detects whether the antibody, typically IgG, can bind tightly to the virus and neutralize it. The extent of virus neutralization determines the antibody titer, and confirmation of acute arbovirus infection, by PRNT, is determined by either a 4-fold change in antibody titer between an acute and convalescent serum or by the presence of antibodies in a single cerebral spinal fluid (CSF) specimen.

If a healthcare provider has a patient with a suspected arboviral disease, they may submit a specimen to the BOL for testing. CSF is the specimen of choice, because it is generally collected when a patient presents with neurological symptoms and the presence of antibodies in CSF is a better indicator of active infection than antibodies from

a serum sample. Antibodies in a serum sample may indicate an active or previous infection or exposure due to the extended period of time IgM lingers in the serum. However, if CSF is not available, paired serum specimens collected at least 2 weeks apart may be submitted with pre-authorization from the Bureau of Epidemiology and Population Health at 517-335-8165. In cases where a serum sample is the only specimen source available for patients who are exhibiting non-neurologic symptoms (fever, fatigue, headache, etc.); specimens from these patients may be sent to a commercial laboratory for testing.

Specimens can be shipped to the BOL on cold packs or at ambient temperature, although cold packs are recommended during the warm summer months. The turn-around-time for IgM testing is approximately 1-2 weeks with most results available within one week. If indicated, PRNT testing can take up to 3-4 weeks.



Photograph courtesy of:

Michigan Department of Health and Human Services, Bureau of Epidemiology and Population Health, Emerging and Zoonotic Infectious Diseases Section

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# Eastern Equine Encephalitis -"EEE"

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*Pictured above:*

*A BOL microbiologist examining PRNT plates in the Biosafety Level 3 (BSL-3) laboratory*

*Pictured Below:*

*PRNT plates—Arrows point to the “plaque formations” typically seen in infected cells*

*Photographs courtesy of the Bureau of Laboratories*



*References:*

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[https://www.michigan.gov/emergingdiseases/0,4579,7-186-76711\\_77442---,00.html](https://www.michigan.gov/emergingdiseases/0,4579,7-186-76711_77442---,00.html)



## Exp!ore Lab Science Exhibit Wins Award

**Author: Sandra Lenneman, MSA, MT(ASCP)SBB, K-12 Outreach**

Exp!ore Lab Science was again voted the favorite exhibit to visit by children of the Michigan Department of Health and Human Services (MDHHS) employees, during the annual “MDHHS Take Your Child to Work Day,” held in August 2019.

Exp!ore Lab Science, is the K-12 science education program sponsored by the Bureau of Laboratories (BOL). The program offers science demonstrations as requested by schools and a website, [www.michigan.gov/explorelabscience](http://www.michigan.gov/explorelabscience), filled with fun science activities for K-12 grade students. The goal of the program is to introduce children to laboratory science and promote laboratory and public health careers.

This year’s theme was “Camp MDHHS.” The Exp!ore Lab Science exhibit offered 5 engaging activities for all participants.



*Giardia lamblia*, which causes Giardiasis, a severe stomach illness with diarrhea. Students could make a Shrinky Dink model of their favorite camping nuisance– a bat, a tick, or a Giardia cell. The Shrinky Dink model was connected to a key chain to be used as a decoration for their backpack or to serve as a reminder about the wildlife or hazards they might encounter while camping.

“Make Your Own Bug Spray,” using essential oils, introduced the participants to a natural way to repel ticks, fleas, and other insects. The children learned which essential oils were the best repellents to use for insects commonly found in Michigan.

Each participant had the opportunity to make their own unique bug spray mixture. Laboratory volumetric measuring and transfer pipetting, for each ingredient, were some of the key scientific techniques used to create the bug spray.



Bats, ticks, and other camping nuisances were on display. All observers were able to examine different types of bats found in Michigan that could carry the rabies virus. Using a stereoscope, interested participants could take a close look at different kinds of ticks and identify the tick that could transmit Lyme’s Disease. The children were also introduced to a parasite found in unsafe drinking water called



A popular camping treat is the s’more made over a campfire. With a pizza box, aluminum foil, plastic wrap, and black construction paper along with some artificial sunshine, the Exp!ore Lab Science team demonstrated how to make s’mores using a solar oven to melt the chocolate and soften the marshmallows.

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# BOL Exp!ore Lab Science Program

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The last exhibit was the Drinkable Density Tower. A tasty red, white, and blue drink was made from different flavored Gatorades. Since every liquid has its own density based on



sugar content, the Gatorade with the highest sugar content remained on the bottom of the glass, with subsequent flavors resting on the layer with the most sugar content. Using the properties of density as the scientific concept, along with a few graduated cylinders, and the art of accurate measurement, the children created a refreshing red, white and blue drink.

The BOL and Exp!ore Lab Science hopes to share more hands-on activities to engage future laboratory scientists during the next “MDHHS Take Your Child To Work Day.” For more information about the Explore Lab Science Program visit our website at [www.michigan.gov/explorelabscience](http://www.michigan.gov/explorelabscience).

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Editor: Teresa Miller

